

The origin of biliary strictures after liver transplantation: Is it the amount of epithelial injury or insufficient regeneration that counts?

Negin Karimian, Sanna op den Dries, Robert J. Porte*

Department of Surgery, Section of HPB Surgery and Liver Transplantation, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Biliary complications continue to be a major problem after orthotopic liver transplantation (OLT). The incidence of biliary complications varies between 10% and 40% in different series and this type of complications is associated with frequent hospital admissions and high morbidity and mortality rates [1–3]. Among the variety of biliary complications that can occur after OLT, bile duct strictures are of the most concern. Bile duct strictures can be classified as anastomotic strictures (AS) or non-anastomotic strictures (NAS). Solitary strictures at the biliary anastomosis have been reported in 9%–12% of the patients [4–6], and NAS have been reported in 1%–20% of patients receiving a liver from donation after brain death and in up to 30% of patients receiving a liver from donation after cardiac death (DCD) [4,7,8]. NAS may occur in the extrahepatic donor bile duct as well as the intrahepatic bile ducts, but they are usually limited to the larger bile ducts. NAS may be accompanied by intraductal biliary sludge and cast formation.

For many years researchers have been trying to understand the underlying mechanisms of AS and NAS. Current evidence suggests that AS are mainly related to the surgical technique and local ischemia of the distal bile duct stump, leading to fibrotic scarring of the anastomosis [1,6]. The etiology of NAS is thought to be multifactorial and three relevant types of biliary injury have been identified as a potential cause of NAS: ischemia/reperfusion related injury; immune-mediated injury; and cytotoxic injury caused by hydrophobic bile salts [1,9]. Current understanding of the occurrence of these types of biliary injury is that they originate mainly after transplantation of the liver. Depending on the severity of bile duct injury, the healing process may lead to scar formation and subsequent stricturing of the affected bile duct segments. Thus far, it remains unclear as to which extent bile ducts of donor livers are injured already before transplantation.

In this issue of the *Journal of Hepatology*, Brunner *et al.* describe a clinical cohort study including 79 liver transplant procedures in

which biopsies were taken from the distal end of the extrahepatic bile duct of the donor liver at the time of organ retrieval and during transplantation [10]. Biopsies were evaluated by light microscopy and immunofluorescence to determine the amount of biliary epithelial injury, using a self-developed semi-quantitative bile duct damage scoring system. The investigators subsequently correlated the grade of biliary injury with the occurrence of biliary complications after OLT. The most striking finding in this study was the high percentage of donor livers with severe injury and loss of biliary epithelium in the distal bile duct before implantation. Of all biopsies taken at the end of cold preservation, 86% had signs of bile duct epithelial cell loss. The degree of bile duct injury increased only slightly after reperfusion of the graft. Few clinicians working in the field of clinical liver transplantation are aware of the fact that the vast majority of donor livers have severe bile duct epithelial injury already prior to implantation. Interestingly, Brunner and colleagues also found that their histological bile duct damage score can be helpful to predict development of bile duct complications after transplantation.

This study is in accordance with another recent clinical study on donor bile duct histology published by Hansen *et al.* [11]. In a cohort of 93 liver transplant procedures, these investigators also performed histological evaluation of donor bile duct biopsies. In this study, biopsies were taken after graft reperfusion, before constructing the biliary anastomosis. This research group described another histological injury grading system that, in addition to biliary epithelium loss, includes the degree of injury of the peribiliary vascular plexus (arteriolonecrosis and thrombosis) and necrosis of the bile duct wall stroma. Major bile duct epithelial loss was observed in 88% (77/93) of the cases, a percentage that is strikingly similar to the 86% of biliary epithelium loss observed by Brunner *et al.* The results from the study by Hansen and colleagues suggested that the presence of arteriolonecrosis in the bile duct wall is strongly associated with the development of biliary strictures after transplantation [11].

These two studies not only provide evidence that histological assessment of the donor bile duct may help predict the occurrence of biliary strictures after transplantation, they also offer additional perspective from which to view the pathogenesis of these strictures. The high percentage of donor livers with severe

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* Corresponding author. Address: Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. Tel.: +31 503612896; fax: +31 503611745.

E-mail address: r.j.porte@chir.umcg.nl (R.J. Porte).



Editorial

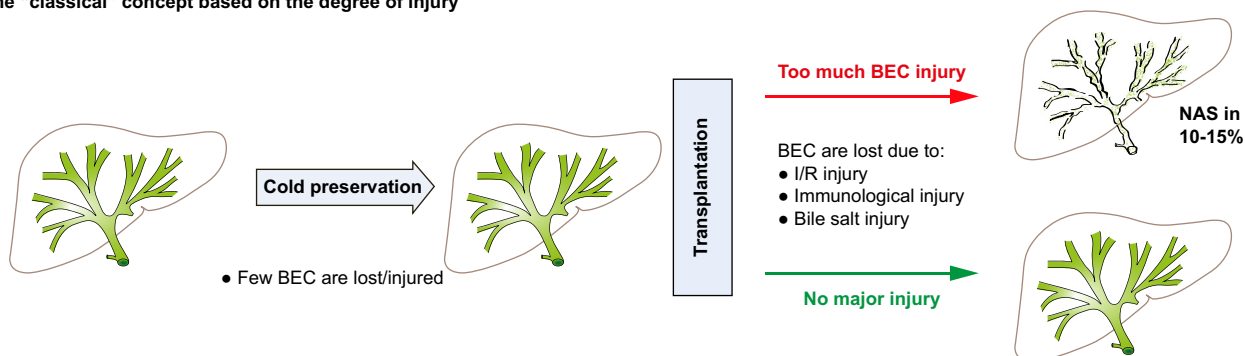
biliary epithelial injury before transplantation is striking. In fact, many extrahepatic donor bile ducts appeared to be nothing more than a scaffold of connective tissue without any epithelial lining of the lumen. With this knowledge, it is a surprise that not every donor liver develops biliary strictures after OLT. The vast majority of donor livers already have severe bile duct injury prior to transplantation, yet “only” 10%–20% develop NAS or AS, it could very well be that the critical factor that determines whether a graft will develop NAS or not is insufficient biliary regeneration rather than the initial biliary injury. This new perspective from which to view the pathogenesis of biliary strictures is summarized in Fig. 1.

The study by Brunner *et al.* [10], as well as the recent paper by Hansen *et al.* [11], opens interesting new avenues for further research. Firstly, the studies show that current methods of organ preservation are far from sufficient to protect the biliary epithelium and bile duct wall in the time period between organ procurement and transplantation. It is possible that current organ preservation fluids used to flush the bile ducts during procurement are suboptimal to preserve the biliary epithelium. Alternatively, it might be that the current preservation method based on cooling is not the best way to protect the biliary epithelium. The only way to eliminate the need for cooling down an organ during preservation would be normothermic, oxygenated organ perfusion. Our group has recently demonstrated that normothermic,

oxygenated perfusion of human donor livers is technically feasible; however, more research in this area is needed to determine whether this will also result in better preservation of the biliary epithelium [12].

Secondly, the papers by Brunner *et al.* and Hansen *et al.* should be seen as a stimulus to study in more detail the processes underlying regeneration of the biliary epithelium. Apparently many livers have adequate and timely restoration of their biliary epithelium, as these livers do not develop biliary strictures despite massive epithelial loss prior to transplantation. Unfortunately, the mechanisms of biliary epithelium regeneration in the larger and extrahepatic bile ducts are not very well known. Most previous research has focused on the smaller, intrahepatic bile ducts and ductules. Studies have suggested a role for the proliferation of remnant biliary epithelial cells, but bipotent stem cells in the canals of Herring (oval cells), bone marrow derived stem cells, and peribiliary glands could be involved as well [13,14]. If the mechanisms of successful reepithelialization of the large and extrahepatic bile ducts are better understood, therapeutic strategies to stimulate regeneration in all livers could potentially be developed. For example, preconditioning of livers to stimulate regeneration of biliary epithelium before transplantation by flushing the bile duct with growth factors or mesenchymal stem cells is an interesting option that needs further exploration.

A The “classical” concept based on the degree of injury



B The “new” concept based on insufficient regeneration

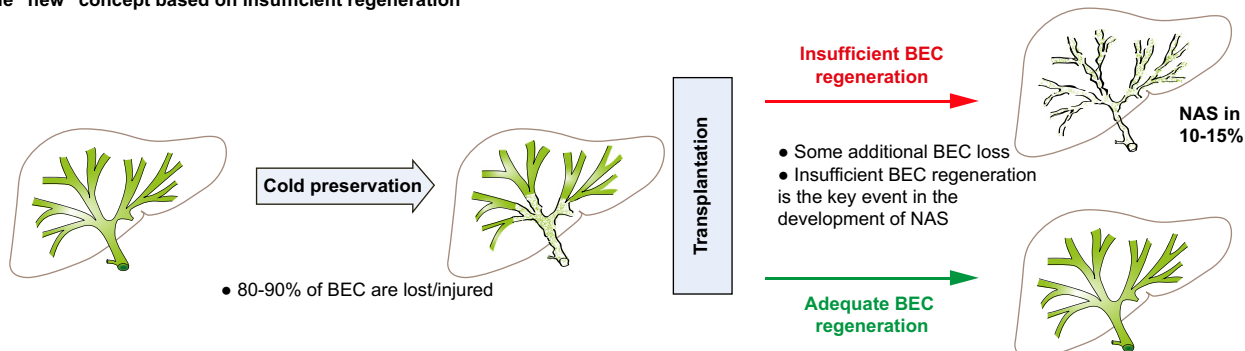


Fig. 1. Schematic presentation of the changing perspective on the pathogenesis of biliary strictures after liver transplantation. (A) The “classical” concept based on the degree of biliary epithelial injury. Few biliary epithelial cells (BEC) are damaged and lost during cold preservation and most part of biliary damage occurs after transplantation due to reperfusion injury, immunological causes, and hydrophobic bile salt toxicity. (B) The “new” concept based on insufficient regeneration of the biliary epithelium. Biliary injury and cell loss are almost universally present in all donor livers. Yet, only a minority of livers develop biliary strictures, suggesting that regeneration of the biliary epithelium is rapid and successful in most donors livers. The key question that arises from this novel perspective is: “What determines successful regeneration of the biliary epithelium and how can we stimulate adequate regeneration in all livers?”.

Thirdly, there is a need to develop non-invasive methods that enable assessment of the biliary epithelium during organ preservation and before implantation. Histological examinations can only be performed by taking biopsies from the distal end of the donor bile duct. It is impossible to obtain biopsies from the more proximal or intrahepatic bile ducts without risk for the graft and recipients. An attractive alternative could be the development of molecular imaging techniques using near-infrared fluorescence that allow a non-invasive assessment of the biliary epithelium. If such molecular imaging techniques are combined with visible light cholangioscopy, this could provide an intraoperative tool for surgeons to judge viability of the biliary tree prior to transplantation [15].

This also touches on a limitation of the study by Brunner *et al.*: specimens were only taken from the distal free margin of the extrahepatic bile ducts. It remains unknown whether injury detected in these specimens is representative of the epithelial lining higher up in the biliary tree. In addition, the series reported by these investigators did not include DCD livers, and it would be very interesting to know the type and degree of bile duct injury in that type of donor livers prior to transplantation.

In conclusion, the study presented by Brunner *et al.* [10], together with the recent data published by Hansen *et al.* [11], gives important new information on preservation injury of the bile ducts in human liver transplantation. These studies provide new perspective from which to view biliary injuries and the development of strictures after transplantation. If loss of the biliary epithelium and injury of the bile duct wall are so universally present in human donor livers, yet (fortunately) only a minority develop biliary strictures after transplantation, an important new question that arises is: "Why does regeneration of biliary epithelium fail in certain livers and how can we stimulate the regenerative capacity of the biliary epithelium after OLT?"

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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